

FAQs

RetroNectin™

1) How does RetroNectin® co-localize virus near cells? Where does it bind to the virus particle? Does RetroNectin® work for retroviruses packaged with different envelopes (Eco, Ampho, VSVG pseudotype)?

RetroNectin® is a ~63 kDa protein of (574 amino acids) that contains a central cell-binding domain (type III repeat, 8,9,10), a high affinity heparin-binding domain II (type III repeat, 12, 13,14), and CS1 site within the alternatively spliced IIIICS region of human fibronectin. Enhancement of retroviral-mediated gene transfer into mammalian cells by co-localization of the retrovirus particles and target cells is accomplished on the recombinant human fibronectin CH-296 chimeric molecules by the direct binding of retroviral particles to sequences contained in the heparin-binding domain II and cell adhesion to sites(s) for VLA-4 and/or VLA-5.

RetroNectin® works for both ecotropic and amphotropic retroviruses. Moreover, this product is useful for retroviruses with a GALV type envelope. In addition, Takara has preliminary data that RetroNectin®-coated plates are also effective for transfection using VSVG (Vesicular stomatitis virus G) pseudotyped retrovirus.

2) The product manual states that virus should be added to the RetroNectin®-coated plates and incubated for 3-5 hours (Step III-2.1, page 4) . Are all virus envelope-types stable for this length of time (3-5 hours) during this incubation? I'm worried that my virus will be dead at the end of this incubation. Does this time period also apply for pseudotype virus?

Takara has verified that ampho, eco and GALV envelope types are stable for a 4-6 hour incubation (sometimes longer). Because pseudotype virus is assumed to be even stronger than the above virus types, a 4-6 hour incubation is also acceptable for this virus type.

3) What type of plate can I use with RetroNectin®?

Non-coated tissue culture plates or dishes should be used for coating with RetroNectin®. Takara has tested both coated and non-coated plate types and found that coated plates had only about 1/5 the efficiency of non-coated plates. We recommend using a 6-well BD-Falcon (# 351146) plate available through Fisher Scientific (Fisher part # 08772-49).

4) How often can reconstituted RetroNectin® be frozen and reused?

Reconstituted RetroNectin® (1 mg/ml) can be used without problems with repeated freeze-thaw cycles up to 5 times. However, avoid excess freeze-thaws and be careful to prevent contamination.

Reconstituted RetroNectin® is stable for one year at -20°C for 1 year or for ~3 weeks at 4°C.

5) How long are precoated RetroNectin® plates stable?

Pre-coated RetroNectin® plates can be stored at 4°C and are stable for 1-2 months (although we recommend using them as soon as possible). Do not store pre-coated plates at -20°C.

6) Does reconstituted RetroNectin® have to be filter sterilized, and if so, what type of filter should I use?

Filtration of the RetroNectin® solution is optional if it is prepared aseptically using sterile water, since RetroNectin® itself is produced under sterile conditions

If you choose to filter your reconstituted RetroNectin® solution, Takara recommends using Millipore SLGV R25 LS or SLGV 025 LS filters. However, we have been notified that these products will soon be discontinued. Therefore, as a substitute, we recommend using either Millipore SLGV 013 SL or SLGV 004 SL filters.

7) Does Takara provide a clinical-grade RetroNectin®?

Clinical-grade RetroNectin® can only be obtained through a Material Transfer Agreement (MTA) arranged specifically between your institution and Takara Bio of Japan. Please contact the following Takara representative in our Japan office to arrange a MTA:

Mr. Kazuhiro Yokota
Business Development
Takara Bio Inc.
Seta 3-4-1, Otsu, Shiga 520-2193, Japan
Email: yokotak@takara-bio.co.jp

8) Can cell-culture bags, like X-fold Baxter bags, be coated with RetroNectin®?

X-FOLD bags can be coated with RetroNectin®. Below is a preliminary protocol which Takara has tested:

For an X-FOLD Bag (85 cm²);

- a) Coat the bag with RetroNectin®(*) overnight at 4°C or for 2 hours at room temperature.
- b) Use at least 9 ml of RetroNectin® solution (20 µg/ml PBS) per bag.
- c) Wash with PBS (30 ml x 3 times).
- d) Preload the Retrovirus vector (4-6 hours).
- e) Wash with PBS (30 ml).
- f) Add cells (1 x 10⁴ cells/cm²).
- g) Incubate at 37°C for 3 days under 5% CO₂.

Please note that this method may not be applicable for other types/brands of cell-culture bags.

9) I know that transfection efficiency is 5-fold higher when non-coated tissue culture plates are used. However, do you have any information regarding the transfection efficiency that can be obtained if coated tissue culture plates are used (even if this plate type is not recommended)? In other words, would it be at all worthwhile to use RetroNectin® on coated plates if coated plates are all I have available to me?

Some experimental data we previously performed showed that transfection efficiency was above 30% when using RetroNectin®-coated non-tissue culture treated plates, but only 1-3% when using RetroNectin®-coated tissue culture treated plates.

The latter is an example using a surface-treated plate. Although we haven't fully tested RetroNectin®-coated plates which are also coated with other reagents (such as collagen), we think that the transfection efficiency will be lower based upon our preliminary data.

However, if you absolutely need a double coat on your plates, please test using the following two coating methods: (1) coat with RetroNectin® first followed by collagen (or other substance), and (2) coat with collagen first followed by RetroNectin®.

If transfection efficiencies are very low in both of the above cases, please try the following method:

Perform the transduction procedure using the proper RetroNectin®-coated non-tissue culture treated plate as described in the manual until Step III-2-1 on page 4. Then, at Step 2-2, discard the virus supernatant, wash the plate and add the target cells. Incubate for 4 hours at 37°C under 5% CO₂, collect the cells and transfer to the collagen (or other substance)-coated plate and continue the incubation for 2-3 days.

10) Is there a way to improve transduction efficiency that is achieved using RetroNectin® up to 60%-70%?

Below are some tips on how to improve transduction using RetroNectin® to around 60-70%. Even without these recommendations, RetroNectin® should give better transduction efficiencies than fibronectin.

- a) Typically we recommend preparing retrovirus supernatant at a high titer ($>10^6$ cfu/mL is desirable). To achieve transduction efficiencies of 60-70%, concentrate your retrovirus solution (e.g. measured titer = 1.15×10^6) to more than 10-fold and use it for preloading.
- b) Pre-load it onto RetroNectin® coated dishes at 200-250 $\mu\text{L}/\text{cm}^2$, and incubate for 4-5 hours at 32°C for good results.
- c) Please be careful not to let the virus-preloaded well dry after discarding the virus supernatant and washing with PBS, at Step 2-2 in "Section III. Transduction Protocol.". Please add your target cells to the growth medium immediately after removing the supernatant. If the virus-preloaded well becomes dry at Step 2-2, the transduction efficiency will decrease remarkably.

11) What procedure do you recommend for dissociating cells from plates which have been transduced using RetroNectin®?

For strongly adherent cells, like fibroblasts, use trypsin prepared in an EDTA/phosphate-buffered saline (PBS) that does not contain Ca²⁺ or Mg²⁺ to dissociate cells.

For weakly adherent (or suspension) cells, use a 0.02% EDTA/PBS solution rather than trypsin to dissociate cells. Use trypsin to remove these cells only if the EDTA/PBS solution is unsuccessful, and realize that trypsin may damage these cells.

Sample procedure for weakly adherent cells:

- a) Following completion of transfection, transfer the supernatant from the plate to a centrifuge tube.
- b) Wash plate with PBS to recover non-adhering cells.
- c) Dissociate adherent cells from the plate using GIBCO's Cell Dissociation Buffer (enzyme free, PBS-based) (Catalog # 13151-014) following the manufacturer's instructions.

- d) Combine all obtained cells in one centrifuge tube, and centrifuge to recover cell fraction.
- e) Rinse cells with HBSS/Hepes twice by centrifugation, and suspend cells in HBSS/Hepes for further use.

12) After cells are dissociated from a RetroNectin®-coated plate using either Trypsin or Cell Dissociation Reagent, are the cells likely to rebind to the same plate? I am performing FACS in a 96-well format (high throughput) and I do not want to switch plates.

Cells dissociated by trypsin or cell dissociation reagent from a RetroNectin-coated plate will likely rebind to the same plate. However, we recommend transferring cells into another plate to read on a FACS (although we realize that this may be somewhat difficult). Takara usually transfers such cells into another plate using a multi-channel pipette.

13) After transduction of cells using RetroNectin®, what method do you recommend to resuspend the cells from the plate? I am using Jurkat cells and I want to stain the CD154 and CD69 cell surface markers and perform flow cytometry. Note that trypsin may degrade my cell surface markers.

For cell lines such as HL60 cells and K562 cells, Takara has verified that cells which have been transduced using RetroNectin® can be easily collected by pipetting them from the RetroNectin®-coated dish. However, for several other cell lines, it may be difficult to collect the cells by pipetting without trypsin treatment.

Takara has not tested resuspension of Jurkat cells with RetroNectin®. In general, if cells cannot be collected by pipetting, we recommend waiting for 24 hours following transduction and then treating cells with trypsin followed by collection. Transfer collected cells into a fresh dish, culture them in suspension for ~2 days, then collect the suspended cells and use them for flow cytometry.